Jan. 11

Dear Josh,

I agree with your comments for the most part. We had predicted that the population would "snap" from one predominant component to another and that the average ratio over a very long time would be that expected from mutation (and specific selection), as in your graph. If each adaptive leap is due to one mutation we would expect a changeover, under the conditions of our experiments, once in 40,000 years on the average. However, our experiments suggest that actually a large number of mutations are involved , since the variance of cycle length is quite small and there appear to be appreciable numbers of the advantageous mutants present when selection first begins. If say 10^4 mutants must accumulate to initiate selection, then in our case some of these would originate from h+ about once in 4 years. But when this occurred it would not result in a changeover, but rather in a small temporary increase in the equilibrium ratio. Of course any sort of effect could result from a sequence of improbable events, a point which is often made in popular articles on statistical mechanics but which we can ignore for practical purposes. A curious feature of periodic selection in our case is that the immediate cause of the adaptive leaps operates in such a way that the increase and decrease of minority mutants is approximately complementary, so that a fairly flat equilibrium is simulated at all times. We know this not only because an equilibrium is in fact observed, but also because we can observe the increase in h+ starting from a small h- inoculum and in other experiments the decrease of marked h+ from equilibrium and we find that these independently observed increases and decreases are in fact approximately (sometimes exactly) complementary. In this respect our periodic selection is subtly different from other non-specific killing agents. Thus both in the (reasonably) long run and in the short run a condition of equilibrium can be brought about by periodic selection.

If you spend a few more hours thought I am convinced that you will be convinced that your statement that periodic selection cannot possibly influence the probability that a single X gene will be + or - is erroneous for practical purposes, although correct if we sum these probabilities over infinite time. The same holds for your statement that periodic selection will simply broaden the dispersion of the distribution of mutants.

I am planning a theoretical paper embodying your suggestions, the above considerations , examples in the literature etc. for the Am. Nat. soon, and I will send you this one before it is submitted. Your suspicions are right about my weakness of character. I won't recall the present one. Its just a preliminary note anyway, and in my opinion no paper can be classed as superb whose content deals with events solely on a populational level. We have changed the authorship to A, S, & Ryan after convincing him that our use of his data warranted this. At first I couldn't tell whether F.J. was just being magnanimous or doubted the validity of our interpretation. It turned out to be the former.

Actually we know a lot more about periodic selection than is stated

in our ms. We now know that colicins are the cause of most, but not all, of the adaptive leaps. Also a population under conditions of stockkeeping rapidly becomes heterogeneous with respect to resistance to its own adaptive potential. Thus if we start a h+/h- reconstruction with stocks "in phase", from 10 to 20% of the indicator population drops out in the first few transfers and the rest drop out at the next adaptive leap some 30 transfers later. Some experiments we have going now indicate (I mean demonstrate) that the many minor fluctuations in +/- ratio which occur between major adaptive leaps are also due to non-specific factors inherent in the system. When we start with h-m- and follow h+ and m+ in the same STs we obtain two equilibria (m+ is a little higher than h+) and the fluctuations in these are uniformly parallel! Cultures from survivors of radiation are often out of phase with the parental culture, as we found out in trying some induced mutants we got from Peg. One of her stable h- mutants is giving a very peculiar result. It remains stable for a (reproducible) period of 20 transfers then produces h+ which rise to a peak then completely disappear within the next 10 transfers, I'll keep you posted on this series. Finally, we suspect that each adaptive leap may be caused by several different kinds of mutations in the major component which are about equally suitable as selectors against the antecedent population without too much interference with each other, I'll give reasons for this if we confirm it.

By the way, I have turned up several mutants in Neurospora which can be most easily interpreted as inhibitor producers. They are in the amycelial component of an ornithineless vs. methionineless-amycelial heterokaryon. The behaviour is as follows: An isolate from UV treated conidia gives conidia which form n essentially normal colonies on minimal, but no normal colonies and more than n amycelials on methionine medium. In two cases these are associated with lethals so that we get normal colonies on minimal but nothing at all on methionine! Size comparisons show that the normal colonies in all cases grow unusually slowly.

In UV effect in Neurosp. I trust the kinetics, which show conclusively that the effect is entirely nuclear except possibly at survival of below .01 . Norman has completed a lot more work on this since we discovered a way to vary the mean number of nuclei at will (by altering the medium). The extrapolates for conidia with 2.3, 4.5, and 5.6 respectively are precisely these numbers except that conidia with 2.3 nuclei show an extrapolate of 2.1 . This was shown by Norman to be due to the uninucleate component they contain which has about twice the inactivation cross-section per nucleus as multinucleate cells. All curves for microconidia go right through one under the conditions of his experiments. He has ruled out shielding as the cause of the increased inactivation cross-section per nucleus in macroconidia. I have shown in two different ways that the probability of homology of recessive lethals is negligible. Therefore the killing of macroconidia must be due to some other effect, and the inactivation cross-section of microconidia (per nucleus) must be larger because recessive lethals are expressed immediately. Last spring I was worried about this interpretation because the kinetics

of recessive lethal production by the heterokaryon method were not first order. I have stopped worrying about this now because in a large number of later experiments the kinetics vary from perfect first order to pronounced maxima with induction periods and declines depending upon uncontrolled conditions of the experiments. There is a beautiful trend throughout. The greater the proportion of mutant nuclei recovered (I have up to 80% now!) the more nearly first order kinetics are observed. A clue to what was happening turned up in an experiment in which I counted mutant nuclei in both components of the same set of isolates. The frequency of isolates with lethals in both components was about three times that of isolates with lethals in one component. Barring unforeseen inhomogeneities in the population, this can only mean that the observed mutants are the residuum of recovery from a more general damage to the genetic material. This initial damage has first order kinetics, and this relates the findings above satisfactorily. Experiments with which you are already familiar show that the non-genetic effect is reparable in the presence of an unaffected nucleus. The problem of UV inactivation of N. conidia seems to be considerably clarified, but I would appreciate any questions you may have. I will have two papers on this finished within about a month and will send them to you.

Turning to your case, I would place a good deal more confidence in the inactivation kinetics than you do, despite the Lwoff effect. As I recall they are the same for the heterozygote as for ordinary K12, and are high multi-hit affairs. This, along with your failure to find balanced lethals strikes me as significant. I would guess that the latter is due to duplicity of each component of the heterozygote. After all, lethal mutations must be more frequent than screenable ones, yet you are not picking them up. A multiplication of genetic sets might result in sufficient resistance of the bacteria to this kind of killing so that cytoplasmic killing would start at relatively high survival and lead to a very high hit number. A kinetic analysis of induced isogenicity might be helpful.

The army is really closing in on me now. Apparently Columbia U. lacks any influence with the medical review board despite many letters of protest including one from the Committee on Govt. aided Researcch explaining that I am now principle investigator on two grants. I learned yesterday that I am still in 1A after review. Of course I wouldn't like to work in BW, but would do so to protect our boys in service from my blundering attempts at therapy. Do you think I should write to Krueger & Braun? Or is there anything else I can do?

Sorry to hear Bussard is homesick, but its natural under the circumstances. I'd be too. KCA IV is very far advanced for 4½ months. I hope we'll see you at CSH this spring if not before.

Best to you and Esther,

Kim

P.S. If you'd have any ideas about this drastic let me know soon, they might call me [illegible]!